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DISCLAIMER

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A HIGH-SPEED AUTOMATED COLONY PICKING MACHINE

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ABSTRACT

We describe a machine that identifies, locates, and picks bacterial colonies from agar culture dishes and arrays them into microtiter plates. Almost all operations are automatic, being based on computer expert image analysis and object recognition and precision computer-driven X-Y-Z placement. Colonies are identified, rejected if they fail a panel of size, shape, and neighbor clearance criteria, and then transferred by tungsten needles from the source plates to the destination microtiter wells. The needles are sterilized by immersion in sonicating heated water or other agents. Each plate is picked at a rate of one colony per 1.4 seconds. The picking machine is coupled to a general-purpose laboratory manipulator robot that loads and unloads the plates from stacks. The time taken for plate changing reduces the overall production rate to approximately 1000 colonies per hour. Tests showed 100% transfer efficiency and 100% sterilization efficiency for *e. coli* colonies. This machine, which has been operational since December 1991, is finding application in large-scale projects associated with the Human Genome Project.

INTRODUCTION

The Human Genome Project calls for the repetitive performance of standard microbiological research procedures on an unprecedented scale. Elucidation of the billionfold number of bases in the human genome will require a commensurate number of operations—many of which, at present, are performed manually. Success in the Human Genome Project hinges inescapably upon laboratory automation on a massive scale.

At present, strategies for human genome research call for cutting the DNA of the organism being studied into tens of thousands of pieces that are cloned into vectors in bacterial or yeast cells. This material is manually plated out on agar dishes and grown up into randomly distributed colonies, each containing a unique fragment of the target DNA. To facilitate processing in such large numbers, these colonies must be "picked" and "arrayed" into rectangular grids of wells in plastic microtiter plates, where they are grown up in an appropriate liquid medium. A genomic "library" is a collection of such microtiter plates containing the DNA of a single individual, or in some cases, a single chromosome.

Colony picking and arraying has traditionally been done manually with toothpicks. Unfortunately, the importance of accuracy is severely challenged by the extreme tedium of the process. Mistakes may not be apparent until much later in the particular experimental sequence, leading to the serious propagation of errors, wasted effort, and the need for constant scrutiny by skilled professionals. Large-scale "colony picks" are rarely entrusted to lowest-grade technicians. Automa-

tion of this task became an appropriate and necessary goal, leading to greater throughput and reduced downstream error.

Successful automated colony pickers have been built at research institutes (3–6) and have also become commercially available in recent months (1–2). At LBL we built a proof-of-concept system in 1990 by programming a general purpose robotic arm to pick with pipette tips (4). At 150 colonies per hour, that system proved far too slow for routine use. On the basis of that experience, we have developed a new picker that is highly specialized for rapid throughput, and can be coupled to the robotic arm for loading and unloading of plates. We describe the new system here for several reasons: first, it has had extensive use since November 1991 at LBL and since May 1993 at Lawrence Livermore National Laboratory; second, it employs a unique mechanical architecture; and third, its image processing algorithms for colony selection are innovative.

MATERIALS AND METHODS

The technical approach to the colony picking machine embodies the following major concepts, as diagrammed in Figure 1 and depicted in Figure 2:

i) machine vision to identify and locate colonies;

ii) programmable X-Y stages to drive the "source" (colony) and "destination" (microtiter) plates beneath needle plungers;

iii) "picking" and "placing" Z-axis motions to insert the needles into the plates;

iv) a carousel to move the needles containing picked colonies from the "source" to the "destination" side of the machine;

v) sterilization, to clean the needles for re-use;

vi) a general-purpose laboratory manipulator robot for loading and unloading plates.

The technical approach was to base the machine on IBM-standard personal computers, and to use existing commercial hardware and software packages as much as possible. In-house programming uses Bioscan Optimas ® and Microsoft QuickBASIC ® for simplicity and flexibility. The machine rate of one cycle per 1.4 seconds is achieved by overlapping picking, placing, and cleaning motions, and by making motions as small as possible.

Picking Details

The detailed sequence of events that leads from the insertion of a source colony plate to the retrieval of an arrayed destination microtiter plate is as follows:

The 100 x 100 mm source plate is placed on the imaging station by hand, where it is held firmly in position by a custom holder and illuminated from below by a fiber-optic flat panel. The video camera is activated and a monochrome image is acquired in the frame grabber. After the colony selection software has processed the image, a certain subset of the total number of objects found

on the plate will be highlighted as candidate "good" colonies. The remainder will be rejected for one of several reasons, as described later. The image processing algorithms incorporate a large amount of experience, but occasionally mis-assign a colony as good or bad. Consequently, the operator has the option of visually inspecting the plate, comparing it with the screen image, and using the mouse to edit the assignments in ambiguous cases. A file of X-Y centroid coordinates of the colonies to be picked is then produced. This data is recorded under a filename that incorporates a three-digit identifier that is automatically incremented for each new source plate.

After being imaged, each source plate is loaded (either manually or automatically, as described below) into a holder on the "pick" station (Figure 3). The data file of coordinates is retrieved, and these are used to position the "pick" X-Y table so that each colony is moved in turn under a spring-loaded tungsten needle. A plunger pushes the needle tip into the colony, coating it with some cells.

There are twelve needles equally spaced around the perimeter of a carousel that rotates in steps of thirty degrees. After each picking motion, the carousel rotates through a step. Three steps after "picking", the same needle finds itself positioned above a well of the destination microtiter plate. This plate is also held in a station on a second programmable X-Y table (Figure 4), so that it may be stepped in an 8 x 12 grid (or multiple, for higher density plates) under the needle position. A second plunger depresses the needle, coated with cells, into a well containing growth medium. A shaking motion of the X-Y table helps to transfer cells from the agar on the needle tip into the liquid. This "placing" step is concurrent with the "picking" step of a needle three positions earlier.

After "placing", the needle continues its journey on the carousel perimeter through the sterilizing station. This consists of a bath of liquid, with heating and sonication available, followed by drying with an air blast. Normally, the liquid used is water heated to 80°C, with sonication enabled. However, other sterilants may be used if called for by specific requirements of the biology. After sterilization and drying, the needle returns to the "pick" position. The twelve needles constantly cycle through the above actions as the carousel turns.

At setup time, the operator selects either manual or automated loading of the picker. In manual mode, which is generally used for short picking sessions, the picking program requests the operator to unload source and destination plates as they are completed and to replace them with new ones. In automated mode, the operator loads microtiter plates and imaged source plates into input stackers accessible by a robot manipulator arm. On command from the picking program, the robot removes a completed plate from the picker and replaces it with a new plate it has prefetched from an input stacker. While the new plate is being processed by the picker, the robot moves the completed plate to an output stacker and fetches the next input plate.

The duties of the operator are thus to load source plates into the imager, to visually edit the colony selection results if necessary, and to load plates into either the robot's stackers or the picker directly.

Imaging Algorithms

The image acquisition and analysis program is responsible for locating the colonies suitable for picking on each source plate, and transmitting a file containing colony centroid coordinates to the

picking control program. The program is based on the Optimas (Bioscan, Edmonds, WA) graphics software. This Windows-based package can be programmed in a high-level manner to perform complex image analysis tasks controllable by custom menus on the PC monitor (Figure 5). A second screen displays the currently acquired image in the frame grabber.

During initial setup, the operator acquires a sample image of a source plate, adjusts contrast and brightness, draws a rectangular region of interest (ROI) on the image, and enters the name and sequence number of the colony coordinate file that will be generated for the first plate. The sequence number becomes a three-digit extension of the filename.

Once setup is complete, the operator images a plate by manually loading it into the holder under the camera and clicking the "Acquire and Process" button, activating the following sequence of events:

i) an image of the plate is acquired and displayed;

ii) background correction is performed to correct for lighting and agar non-uniformities, by fitting a two-dimensional third-order polynomial surface to average gray levels in the ROI and subtracting it from the image;

iii) a global threshold is automatically chosen just below the large background peak in the histogram of gray levels in the ROI (this threshold may be adjusted by the operator if manual thresholding mode was selected);

iv) objects darker than this threshold are identified as candidate colonies, and various size and shape measurements are made on them;

v) a series of size, shape, and neighbor proximity tests are performed as described below, and objects failing one or more tests are rejected;

vi) the centroid coordinates of the remaining "good" colonies are written to a file to be used by the picking machine;

vii) the sequence number is incremented in preparation for the next plate.

At this point the candidate "good" colonies are outlined in red on the screen (Figure 8). If the operator agrees with the assignments made by the program, she simply processes the next plate. If not, she selects the "Edit Results" button and, by comparing the screen assignments to the actual plate, adds or deletes objects from the "good" category by clicking on them with a cursor controlled by the mouse. As each object is selected, the PC monitor displays a diagram of its shape, a plot of the density profile along its major axis, its size and shape measurements, and a list of the reasons, if any, that the program rejected it. This information assists the operator in deciding whether to reassign the object. When editing is complete, the output file is replaced with the new list of "good" colony coordinates.

Much development work resulted in the present algorithms that determine the eligibility of colonies for picking. The tests are designed to select single, round colonies and to reject colonies

grown together. The actual cutoff values for all tests are empirically determined and may easily be changed, as described below.

In the first three tests, the area, aspect ratio (defined as the ratio of length to width of the bounding box parallel to the major axis of the object), and circularity (defined as the ratio of perimeter squared to area), must all be within prescribed limits. The aspect ratio and circularity limits are functions of area, and are looser for small objects than large ones, since small objects have fewer pixels and thus more variability in their measurements.

In the fourth test, the density profile along the major axis of each object is scanned, and objects without a single peak are rejected (Figure 6). This test provides a very positive check against multiple colonies grown together.

The fifth test rejects distinct objects that are so close together that they may actually be touching at gray levels below the chosen threshold. The program establishes a circular "safety zone" about the centroid of each object, with a diameter slightly larger than the object's major axis (e.g., 105% of the major axis). If the safety zones of two objects intersect, both objects are rejected (Figure 7A).

It is possible to have a very small colony so close to a larger one that a needle could nick the larger one when picking the smaller, even though the two safety zones do not intersect. To prevent this, we reject an object if the needle radius about the object's centroid intersects the safety zone of an other object (Figure 7B). Thus the smaller colony is rejected while the larger one is safely picked. For this purpose, the needle radius includes the actual radius of 0.5 mm plus an empirically determined aiming error of 0.21 mm.

The sixth and final test rejects colonies very close to the ROI edges, because they are potentially too close to possible unseen colonies outside the ROI. Hence we reject any object whose safety zone intersects an ROI edge, or whose centroid distance from any edge is less than the needle radius plus aiming error (Figure 7C).

The actual limits for all these tests are contained in a default parameter file that is loaded when the imaging program is started. From the imaging menu it is possible to load different files, each tailored to a specific library type, and to change values in these files with a text editor.

Parameters may be tuned in operation by activating a Microsoft Excel ® spreadsheet that displays scattergrams of aspect ratio vs. area and circularity vs. area for the objects on a series of imaged dishes. These plots show the assignments of "good" and "bad" made by the imaging program, as well as reassignments made by the operator. As new test limits are entered on the spreadsheet, the plots immediately reflect the revised assignments, allowing the operator to choose parameters that minimize false assignments. The updated parameter values may then be uploaded from the spreadsheet to the imaging program.

Hardware Details

This section provides detailed specifications of the equipment used. Components for which a manufacturer is not specified were custom built at LBL.

On the imaging station, the source plate (Nunc Lab-Tek 4021, Naperville, IL) is held by a spring-loaded clip in a custom holder and illuminated from below with a Fostec (Auburn, NY) model 8925 10.2 x 12.7 cm (4 x 5 in) flat panel, coupled via fiber optic cable to a model 8300 incandescent light source. A diffuser sheet on top of the panel helps remove nonuniformities in the field. The monochrome CCD video camera is a Pulnix (Sunnyvale, CA) model TM745E to which we have added a Nikon (Tokyo, Japan) Nikkor 28 mm f/2.8 lens. The high quality of the lens is important for accurately locating the colonies. The camera feeds an Imaging Technology (Bedford, MA) PCVision Plus frame grabber with 640 by 480 8-bit pixels, yielding a pixel size of about 0.18 mm square.

On the picker, the source plate is held in a holder identical to that on the imaging station. Both the source plate and destination plate translation tables (Daedal, Harrison City, PA) are open frame tables with 15 cm of travel in the X and Y directions. The source plate translation table is powered by a microstep motor. For high picking accuracy, linear encoders on the table have a resolution of 0.0051 mm (0.0002 in). The destination plate translation table is powered by a half step motor with a resolution of 0.0635 mm (0.0025 in). This lower resolution is more than adequate to target the 9 mm spaced wells in a standard 96 well microtiter plate.

The needle carousel is a 50.8 cm diameter disk with twelve needle assemblies mounted at equally spaced intervals at a 22.9 cm radius from the center of the disk. The rotating carousel is supported by steel bearings at a 19.1 cm radius to prevent tilt of the carousel when the needles are depressed by the plungers. The circumference of the carousel is cut with 396 teeth to match the sprockets of the steel core timing belt that rotates the disk. The carousel is driven by a microstep motor fitted with a rotary encoder with 0.18 degree resolution.

Each needle assembly consists of a stainless steel shaft with a 44.5 mm long, 1.0 mm diameter tungsten rod mounted on the bottom end of the shaft. The tungsten rod is ground flat at the picking end. Since each needle position is individually calibrated, it is important to maintain the integrity of the calibration by preventing the needle from inadvertent bending or rotation. Hence we use tungsten, which is so stiff that it will break before bending. It is not difficult to insert and calibrate a new needle. The needle is constrained from rotating by a set screw sliding in a groove in the shaft.

A semicircular cam above the bath contacts a nylon cap nut on top of each needle shaft and depresses the needle about 25 mm into the bath for four to five seconds as the carousel plate rotates above the custom built sterilizing bath. An ultrasonic transducer (Blatek, State College, PA) is epoxied to the bottom of the 2 liter bath using Torr Seal. The epoxy can withstand temperatures of 100°C; however a cooling fan protects the transducer and the epoxy joint as well. The transducer driver is taken from a Branson (Danbury, CT) 2 liter desktop ultrasonic bath. Ultrasonic agitation is achieved throughout the entire volume of the bath. The entire cleaning unit is removable for maintenance or replacement.

A custom designed unit heats the sterilizing liquid and circulates it through the bath. Liquid drained from the bath is filtered of cell debris before recirculation. Sensors monitor the total liquid volume and evaporative losses are replenished. Vapors are directed away from the colony picker.

As each needle leaves the bath, a computer-controlled solenoid provides a puff of air from a compressed air source, to blow off any residual fluid.

The entire mechanical unit is mounted on a movable cart 1.2 m wide by 0.81 m deep by 0.91 m high (48 x 32 x 36 in). The computer, X-Y table drive modules, and the bath heater unit are located inside the cart. There is a transparent protective cover over the top of the instrument, and the front is open to allow for loading and unloading plates. The robot loader is mounted on a separate detachable cart of similar size that bolts to the front of the picker cart.

Robotic Loader

We have implemented automated plate handling for the colony picker with the addition of a Hewlett-Packard Microassay System ® manipulator arm. This system was used in our original colony picking machine (4). While too slow to serve as a practical colony picker, it has adequate speed for plate loading.

Because the Microassay system's proprietary software is not compatible with the Windows ® operating system on the picking machine, the loader control system runs on a second IBM-compatible computer. The two computers communicate over a standard RS232 link using a simple command and response protocol. The loader can also read a bar code label on the source plate and send an identifier to the picker, so that the correct file containing colony coordinates for that plate is used.

The robot arm and custom stackers are located on their own detachable cart in front of the colony picker cart as shown in Figure 2. The carts are bolted together so as to give precise registration between the two machines. The robot arm loads and unloads colony and microtiter plates on demand from the colony picker, and notifies the picker when the action is complete. While the picker is picking colonies, the robot prepositions new colony and microtiter plates and removes the finished plates to the completion stacks. Prepositioning new plates reduces the distances that the robot must move at critical points in the process and therefore reduces the amount of time for plate exchanges.

Control Software

The software that controls the colony picker system is divided into three main modules. One of these modules, the software for the robot loader, runs on its own computer as has already been described. The other two modules, the imaging and picking programs, run in a time-shared mode under Microsoft Windows ® on a second IBM-compatible personal computer. The picking program runs in background mode, because control of the motion actuators requires little processing overhead. The computer- and operator-intensive image analysis runs in the foreground. Since these two programs are not linked, they could be run on separate computers, but the slight gain in speed would not justify the extra expense.

The imaging software is written in Optimas ®, a high-level Windows-based imaging language. We generated the custom operator menus (Figure 5) using the Microsoft QuickC for Windows Dialog Editor. The picking control program is written in Microsoft QuickBASIC ® for ease of development and maintenance. It runs in its own window as a "non-Windows application", and

the operator controls it with keyboard commands. To provide a more convenient mouse-driven operator interface, we are converting this program to Microsoft Visual BASIC ®.

The picking control program produces ASCII code strings that are sent to the motor control logic boards to produce the various motions. Firmware on these boards controls the actual motor kinematics. A number of BASIC subprograms produce specific low-level actions; these are linked together in the main program that processes complete plates. Much attention was paid to optimizing the timing by interleaving motions, and by preprocessing the list of colony coordinates with a piecewise path optimizer to minimize the travel from one target to the next. Mechanical crashes are rigorously avoided by confirming that all axes have stopped moving before instigating the next action.

Calibration

Before colony picking can begin, two mechanical calibration procedures must be performed. The first of these establishes a mapping between the coordinate system of the imaging system and that of the picker, so that colony image coordinates may be translated into mechanical X-Y coordinates for picking. This calibration is done by means of a 100 x 100 mm transparent calibration plate that has three accurately inscribed reference marks forming a right angle with edges 50.8 mm (2.00 in). Using a calibration submenu in the Optimas imaging program, the operator images this plate and generates a calibration file containing the coordinates of the three marks in image space.

The plate is then moved to the source plate holder on the picking machine, where the operator moves the source X-Y table under control of a calibration program so that each reference mark in turn is visually located under needle #1 as accurately as possible. (Needle #1 is identified by its location in relation to an optically-sensed index hole in the perimeter of the carousel.) The program records the X-Y encoder positions of the three reference marks. Now that the positions of the marks are known in both image and mechanical space, the translational and rotational mapping between the two systems can be made. This calibration is repeated if the camera is moved relative to the imaging source plate holder in any way, or if critical mechanical components on the picker are replaced.

The second procedure calibrates the tip positions of the twelve needles. This calibration relaxes the need for precise mechanical alignment of the individual needles in their shafts, and is important when picking small or closely spaced colonies. The calibration program on the picker allows the operator to visually position each needle in a 1.27 mm diameter hole in the calibration plate. The X-Y encoder positions are recorded in a file that is used to compute the tip position of each needle during picking. When a needle is replaced, the calibration can be redone for that needle alone in a few minutes.

RESULTS

Our system reached operational status in December 1991, when it was first used to pick a *Drosophila* P1 library of 10,800 clones in 16 hours. The imaging software then in use was slow, difficult to use, and could perform only very limited size and shape tests. The introduction of the Optimas software in May 1992 resolved these problems. As of July 1993, the machine has been

used to pick six libraries of several thousand clones each. In the largest of these, about 16,000 colonies were picked over three days. All picks to date have used *e. coli* with cosmid or P1 phage vectors containing inserts of *Drosophila* or human genomic DNA or human cDNA.

A second picker was installed at the Human Genome Center at Lawrence Livermore National Laboratory in May 1993. This system is identical to the first machine except that it has no robotic loader. As of July 1993 it has been used to pick a 14,000 clone human chromosome 18 cosmid library in two days.

Picking Performance

The picking machine achieves a maximum rate of 1 colony per 1.4 seconds, or 2500 colonies per hour. We experience a net throughput of about 1000 colonies per hour when the time to change plates is considered. A single operator can handle both imaging and picking at the same time in "pipeline" mode, in which each colony plate is imaged, then transferred by hand to the picker where it is picked while the next plate is imaged. Since imaging is faster than picking, it does not affect the overall throughput rate. Microtiter plates are filled with growth medium with a bench-top machine prior to a picking run.

Imaging Performance

Figure 8 illustrates the results of fully automated imaging on a poorly plated colony dish. Colonies chosen for picking by the program have darkened outlines and major axes. With no intervention from the operator, the program has successfully eliminated all colonies grown together or nearly touching. The small round bubble at lower left was also rejected because its two edges produced a double peak in its density profile. Processing time on a 33 MHz 486 PC was twelve seconds.

Biological Testing

There are two tests necessary to determine that the instrument is working properly as a colony picker: transfer efficiency and sterilization efficiency. We perform both tests on each new organism before use in the picker. Transfer efficiency is tested by observing growth in microtiter wells after picking colonies. When early testing revealed less than 100% inoculation, we added a "dithering" motion to the destination X-Y table to shake cells loose from the needles. This resulted in 100% inoculation.

Sterilization efficiency is tested by picking colonies, sterilizing the needles, then inoculating new microtiter wells without picking additional colonies. Our tests found that hot water is more effective at sterilizing *e. coli* than either 70% or 100% ethanol. Ethanol at room temperature did not kill some strains we tested. Furthermore, ethanol dries any agar stuck to the needles, ultimately leading to a buildup that degrades both sterility and picking accuracy. On the other hand, hot water dissolves the agar and removes any accompanying cells, which then are removed by the filtration system. In our tests, 80°C water sterilized 98% of the needles without sonication, and 100% with sonication.

DISCUSSION

There have been two distinct types of use for the colony picker. The first involves large scale picks of several thousand colonies, as described above. The more common use is for picks of 500–2000 colonies for investigators in our laboratory.

While the large picks are infrequent, they justify travel from distant locations to a center with automated picking capabilities. Our machine has been used in this fashion by Washington University (St. Louis, MO), Lawrence Livermore National Laboratory (Livermore, CA) (before installation of their system), and Amgen (Thousand Oaks, CA). Once the current software was in place, the ease with which the instrument can be used was demonstrated by turning over operation of the instrument to technicians from those sites after short training sessions.

To enhance image processing speed, we have chosen simple algorithms for colony selection, but always use methods that are conservative. It is clearly preferable to miss a good colony than to pick commingled growth from two colonies. As an illustration, the use of a circular safety zone to screen out near neighbors sometimes results in rejection of good colonies that lie beside long streaks. We are willing to accept this limitation in the interest of speed, because it errs on the conservative side.

The performance of the image analysis is of course highly dependent on the preparation of the colony plates. Consequently, we attempt to plate the colonies as uniformly as possible, and prepare several trial plates at different concentrations before plating out an entire library. The goal is to minimize the number of colony plates to be stored and handled, yet maintain good clearance between neighboring colonies. We have found that between 80 and 120 colonies per 100 x 100 mm plate yields the best results.

Our operator interface for the imaging program is designed to be both simple and flexible. Once setup is complete, a colony plate image may be completely processed by clicking a single button. If the results are unsatisfactory, other options are readily available such as editing the good/bad classifications directly on the image, or readjusting the contrast, brightness, threshold, or colony selection parameters and reprocessing the plate. In addition, the output file name, directory, and sequence number are easily changed on the main menu, and various calibration routines are quickly activated from a submenu. We are rewriting the software for the picking machine in Visual BASIC with the same design criteria in mind.

Although the imaging system currently runs under supervision of the operator, the colony selection algorithms are now sufficiently mature to be run fully automated. Once familiar with the system, operators invariably choose automatic over manual thresholding, and rarely edit the assignments made by the program. For example, during a recent picking run, only seven colony assignments out of a representative sample of 1017 were edited. These all were colonies that the program rejected because of their proximity to the ROI edge. The operator, having the advantage over the program of seeing that no nearby colonies were outside the ROI, accepted them. In an automated system, the loss of those seven colonies would under ordinary circumstances be perfectly acceptable, and if not, they could quickly be picked by hand. More importantly, there were no assignments during this run that were reversed by the operator in the opposite direction, i.e., the program successfully found all the overlapping colonies that should not be picked.

An automated colony picking system like that described here provides several important benefits to a microbiology laboratory. Among these are the accuracy and reliability of the machine, leading to greatly reduced concern about the propagation of errors due to mis-placed colonies. In addition, skilled personnel are made free by instruments such as this to pursue much more interesting tasks. Finally, the capability of rapidly picking tens of thousands of colonies by machine allows the biologist to contemplate new experiments that, for practical reasons, were formerly beyond reach.

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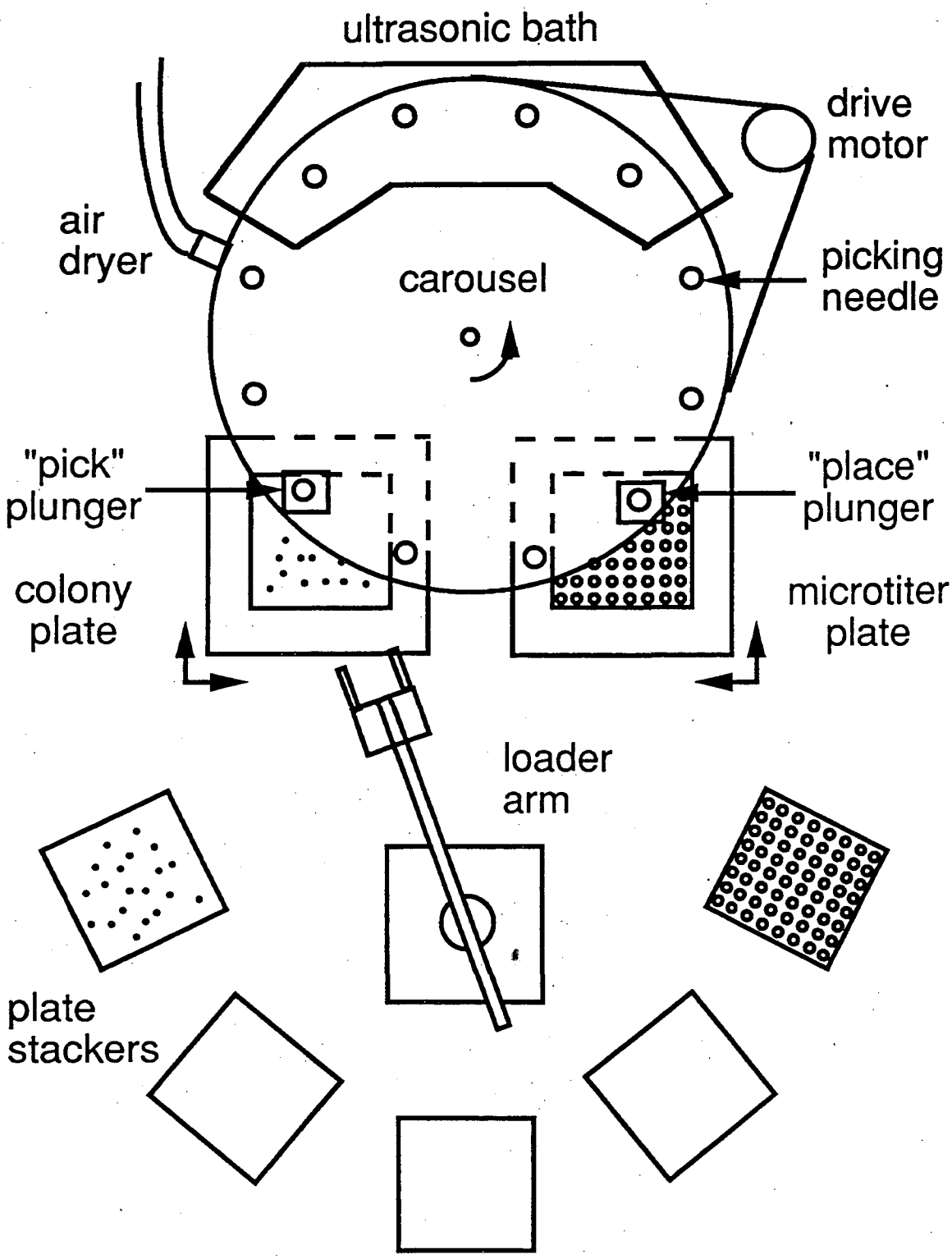

Figure 1. Schematic of the colony picker workstation. Dual X-Y tables simultaneously align a source colony and target microtiter well under fixed "pick" and "place" needle positions. The carousel rotates the needles through a cleaning station prior to another pick cycle. The loader arm concurrently stores old plates and fetches new ones.

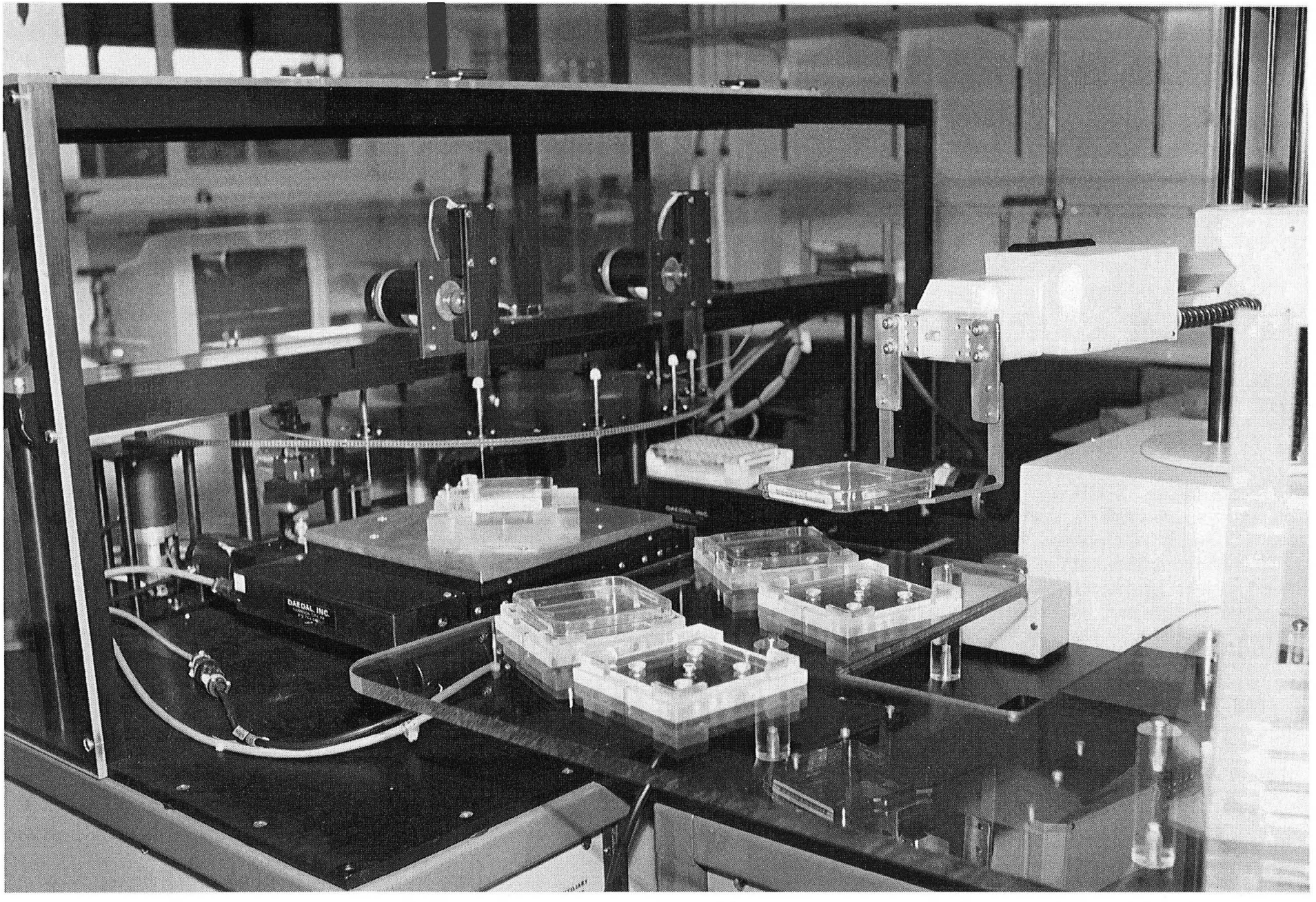

Figure 2. Photograph of the colony picker showing the source and destination X-Y tables below the needle carousel. The robotic loader arm is at right.

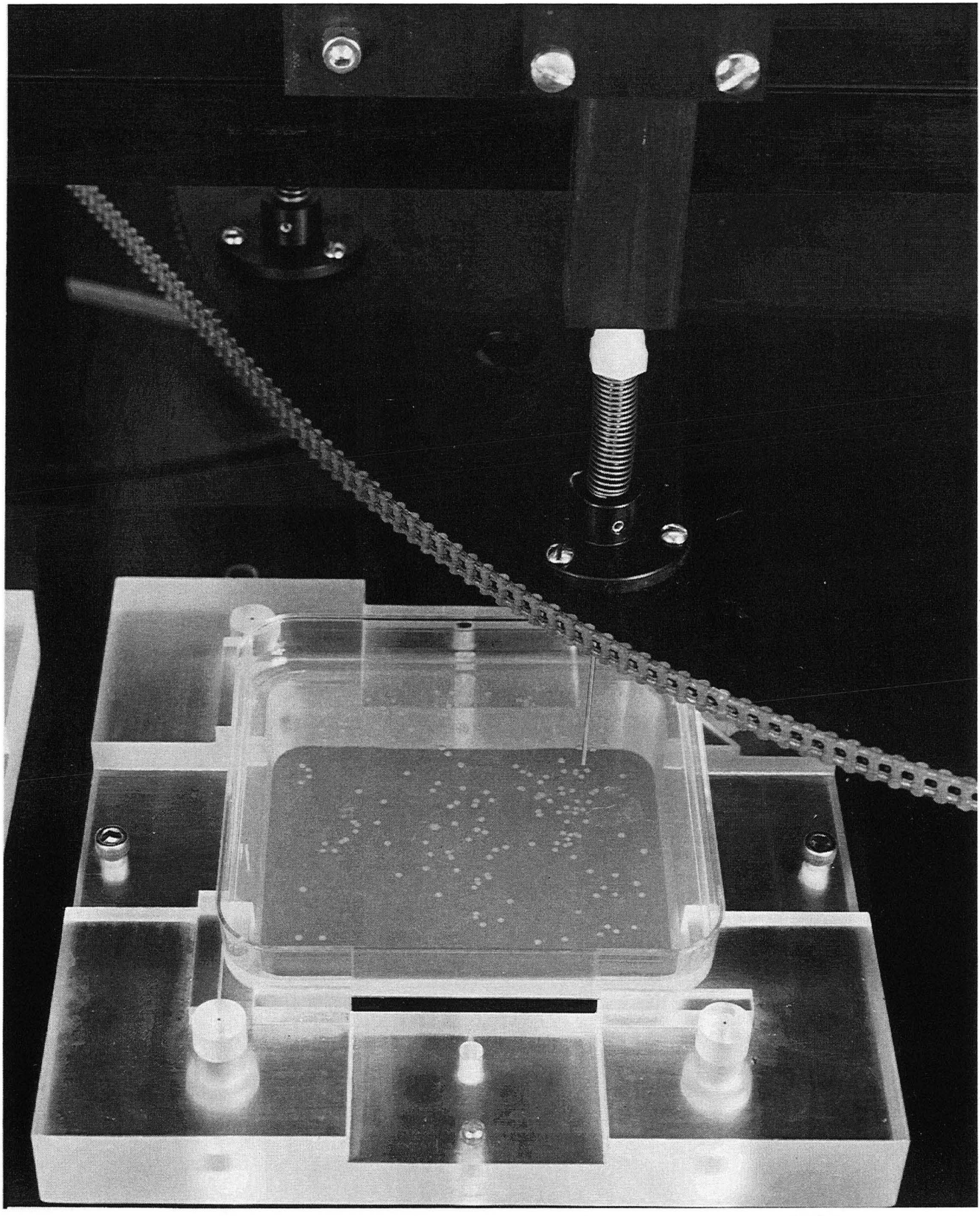

Figure 3. Close-up of a colony plate in the picking station. The spring-loaded needle on the carousel is being actuated by a plunger.

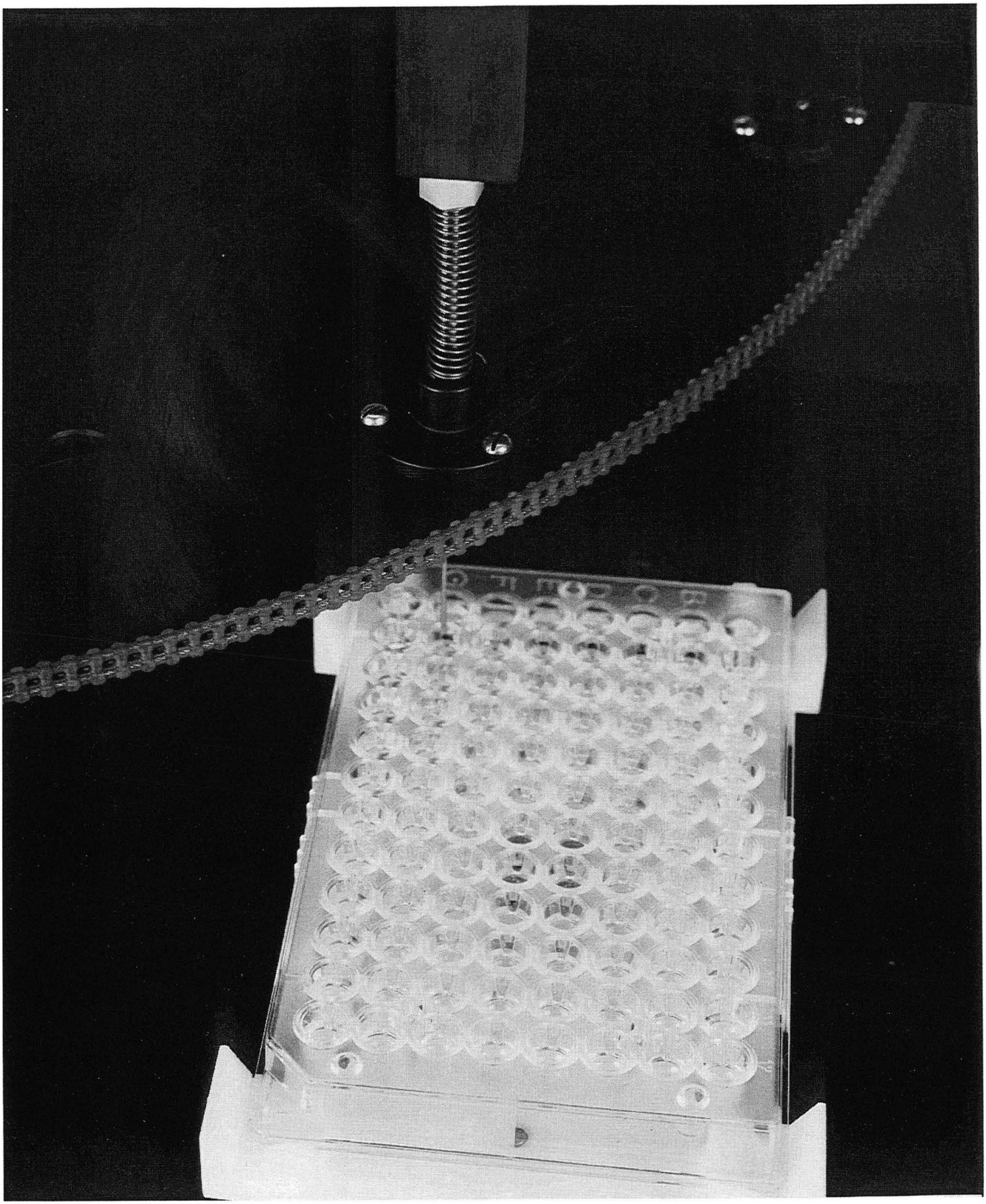

Figure 4. Close-up of a microtiter plate on the placing station. A plunger is pushing the needle into a well.

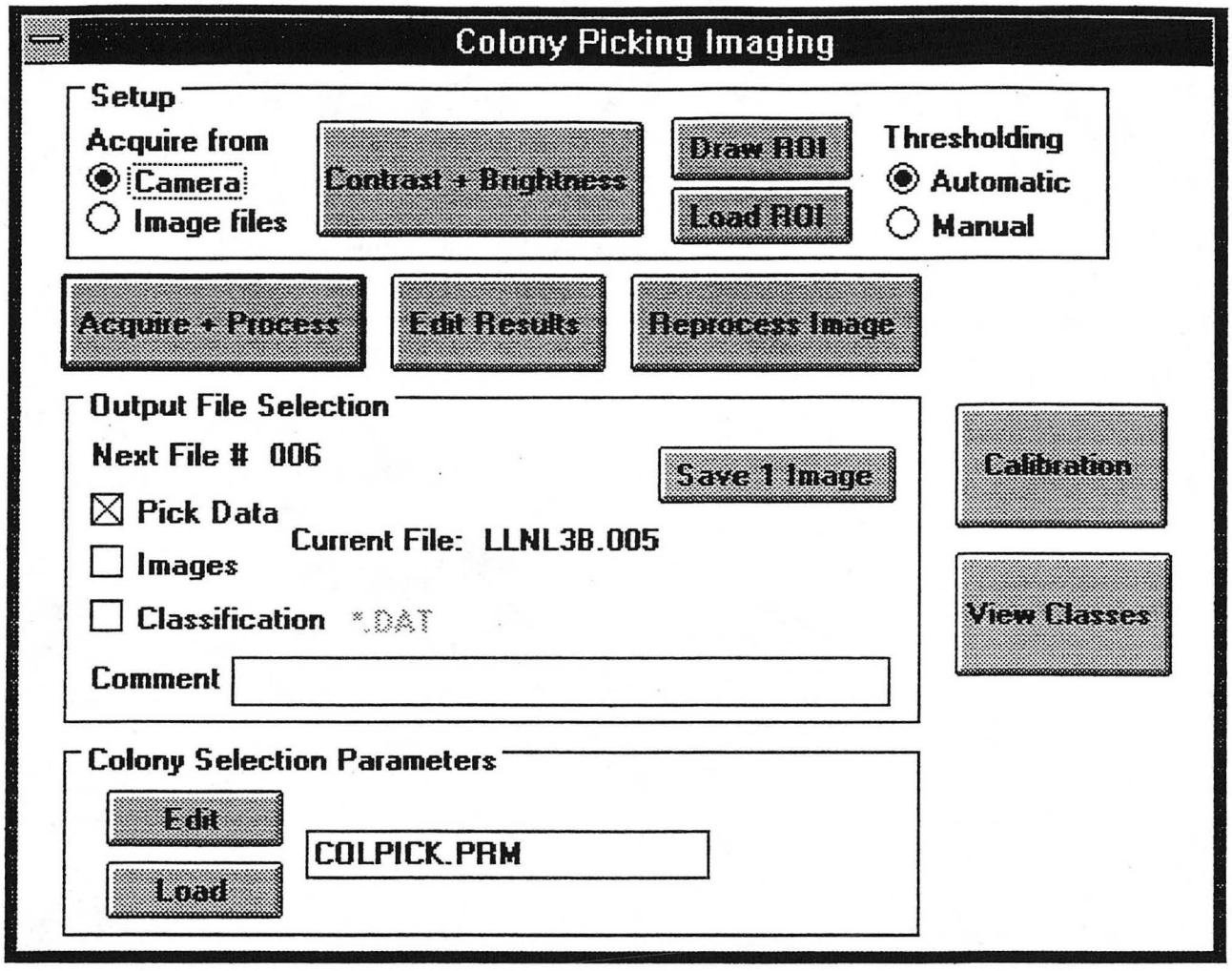

Figure 5. The operator control menu for the imaging program. The Calibration button brings up another menu for imaging system calibration.

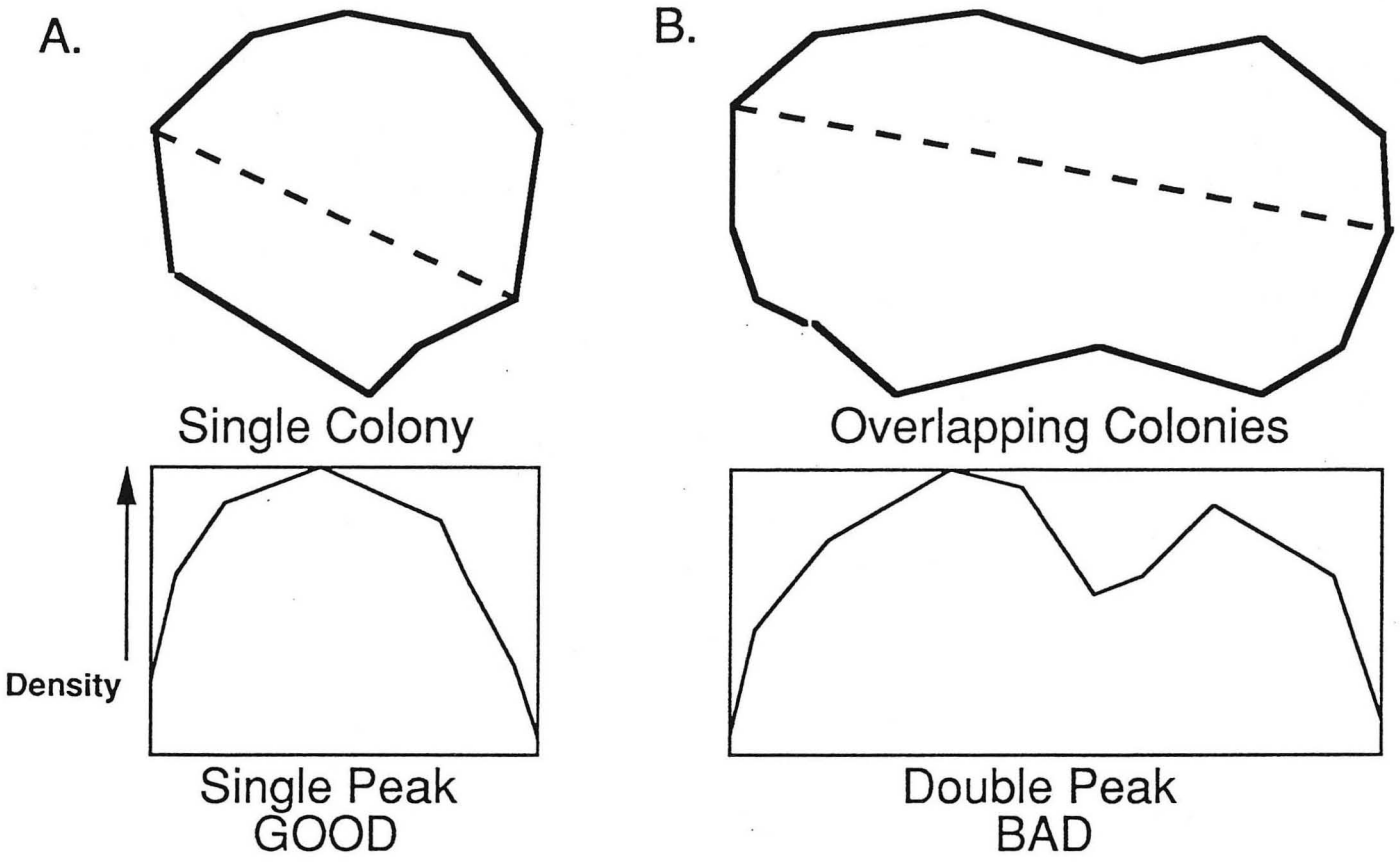

Figure 6. The density profile along the major axis (dashed line) is one of the tests used to distinguish single colonies (A) from overlaps (B).

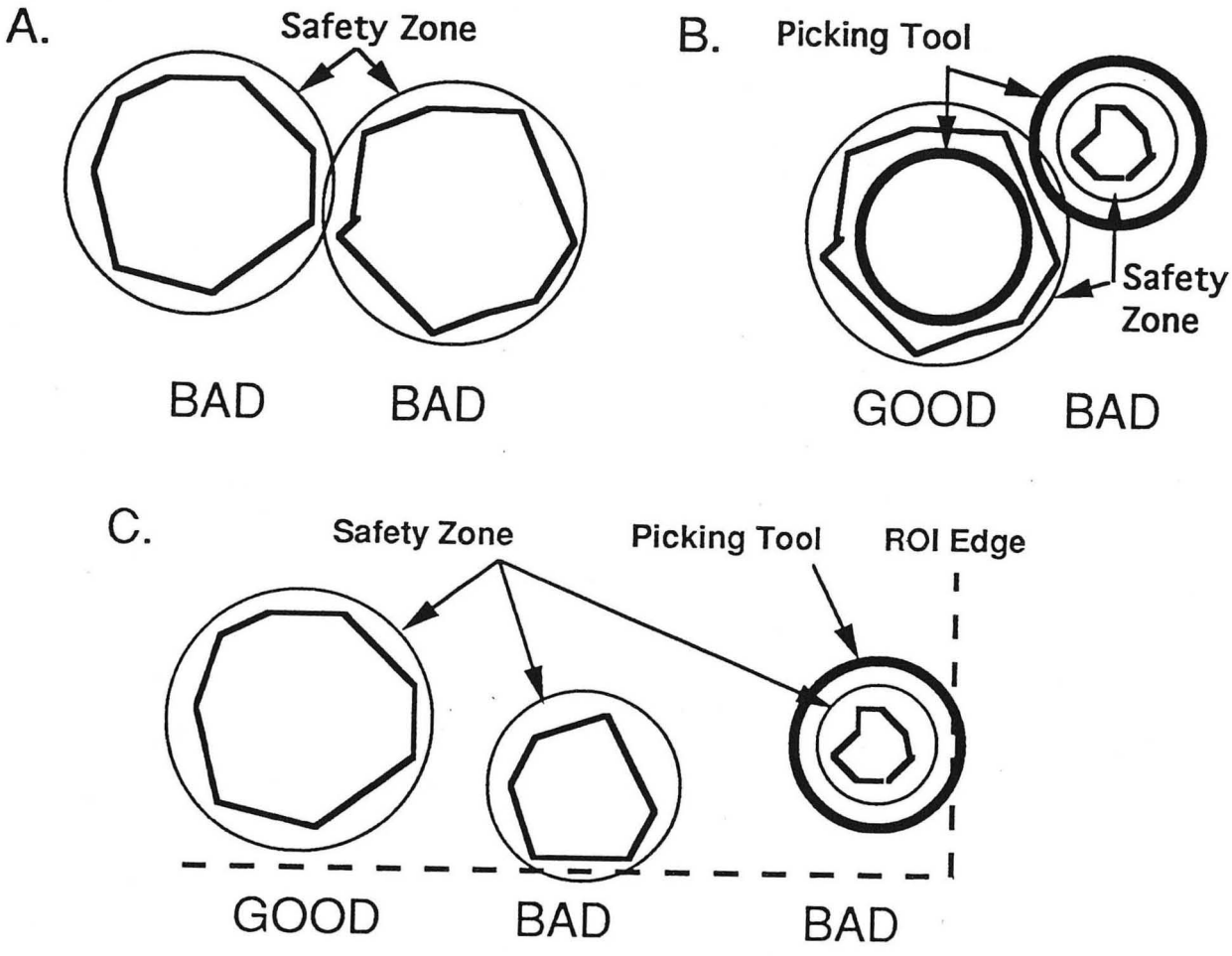

Figure 7. Proximity tests: A) Objects whose circular "safety zones" intersect are rejected. The safety zone of each object is centered at the centroid and has a radius that is proportional to the major axis. B) A small object is rejected if the picking tool would intersect the safety zone of a neighbor. C) Similar tests are made at the edges of the region of interest.

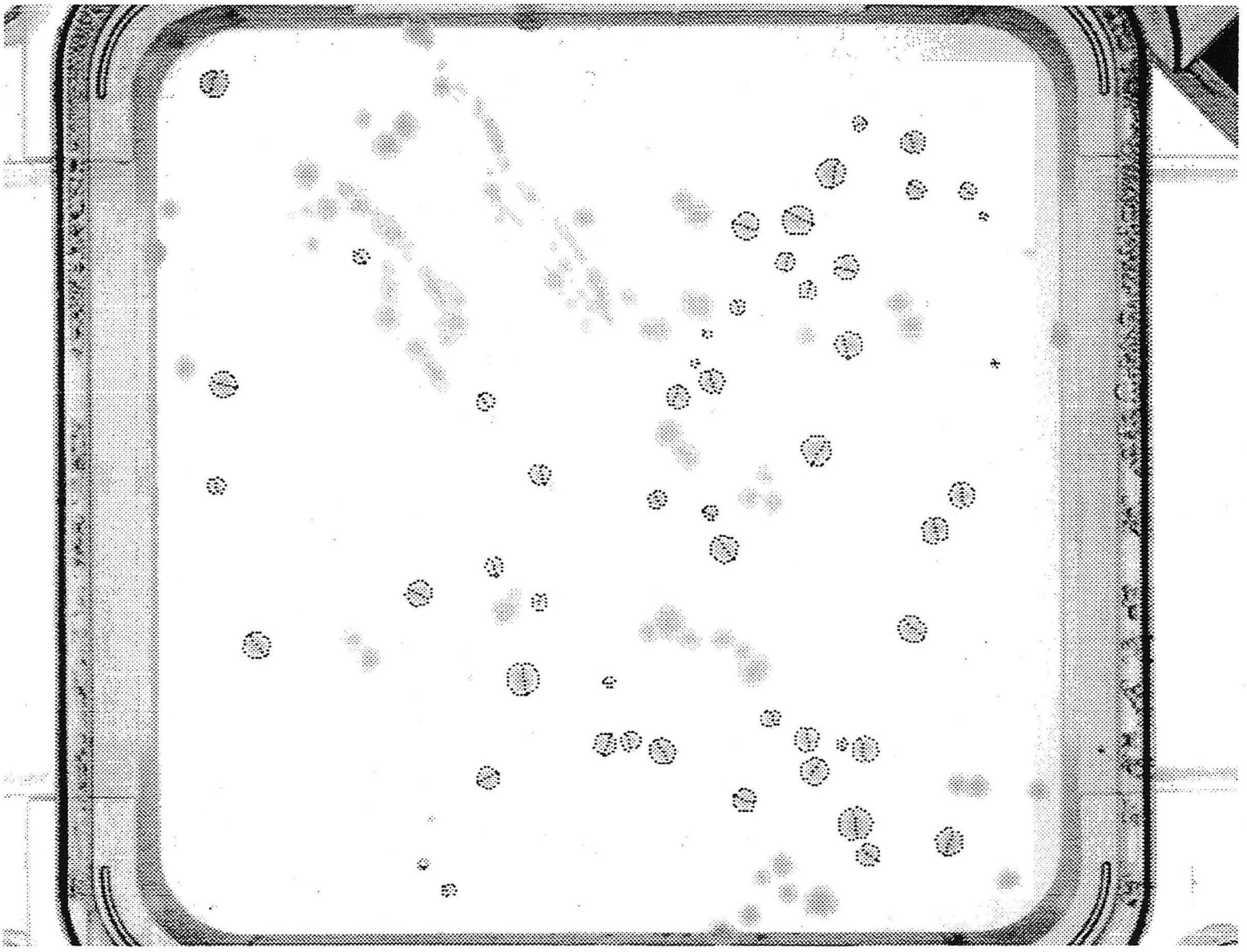

Figure 8. Results of fully automated analysis on a poorly plated dish. The program has rejected objects unsuitable for picking inside the region of interest (dashed box). The remaining good objects have dark outlines and major axes.

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